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RABIES-HYDROPHOBIA.

A STUDY OF FIXED VIRUS, DETERMINATION OF THE M.L.D., VACCINE TREATMENT (HÖGYES, PASTEUR, AND DIALYZED VACCINE), AND IMMUNITY TESTS.*

JAMES GORDON CUMMING.

(From the Pasteur Institute of the University of Michigan, Ann Arbor, Michigan.)

The object of this paper is to present, first, the results of research on fixed virus, i.e., the action of disinfectants, the effect of heat, passage through Berkefeld filters, infectivity, exposure to peritoneal fluid *in vivo*, and dialysis; second, the determination of the M.L.D. of the virus and the results of immunity tests on sets of animals which had been vaccinated by the various methods of antirabic treatment, i.e., Högyes, Pasteur, and a new method, devised in this laboratory, which will be designated the method of dialysis.

Throughout the experimental work, unless otherwise stated, a standard suspension (1–100) of fresh rabic brain in physiological salt solution was employed. The preparation of this standard homogeneous suspension is given in detail under the consideration of the M.L.D.

The following tabulations show the effect of disinfectants on the virus. Their action was determined by injecting 0.5 c.c. of the test suspension intracranially into either rabbits or guinea-pigs. At the termination of each series of injections from the test suspensions the original untreated suspension was injected as a control.

EXPERIMENT 1.

	Exposure	Death from Rabies
Effect of 1 per cent tricresol on virus suspension.......	5 hours	6th day
	7 "	" "
Effect of 1 per cent phenol on virus suspension.......	5 "	" "
	6 "	" "
Effect of 0.4 per cent formaldehyde on virus suspension..	5 "	Lived[1]
	6 "	"
Control...........	....	6th day

EXPERIMENT 2.

	Exposure	Death from Rabies
Effect of 0.04 per cent formaldehyde on virus suspension............	15 minutes	6th day
	30 "	" "
	1 hour	" "
	1.5 hours	7th "
	2 "	8th "
Control...........	....	7th "

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[1] Throughout this paper the word "lived" indicates that the animal was alive two months after the injection.

* Received for publication August 12, 1913.

EXPERIMENT 3.

	Exposure	Death from Rabies
Effect of 0.08 per cent formaldehyde on virus suspension............	1 hour 1.5 hours 2 " 2.5 "	6th day 7th " Lived "
Control............	....	6th day

EXPERIMENT 4.

	Exposure	Death from Rabies
Effect of 0.1 per cent formaldehyde on virus suspension..	1 hour 2 hours 3 "	6th day Lived "

EXPERIMENT 5.

	Exposure	Death from Rabies		Exposure	Death from Rabies
Effect of 0.5 per cent acetone on virus suspension.....	3 hours 6 "	6th day " "	Effect of 0.5 per cent furfurol on virus suspension...	3 hours 6 "	Lived "
Effect of 0.5 per cent acetaldehyd on virus suspension	3 " 6 "	Lived "	Effect of 0.5 per cent salicylaldehyd on virus suspension..................	3 " 6 "	6th day 7th "
Effect of 0.5 per cent benzaldehyd on virus suspension..................	3 " 6 "	" "	Effect of 0.5 per cent anisaldehyd on virus suspension	3 " 6 "	6th " " "
			Control..................	....	" "

In the following experiment the rabic brain tissue was emulsified in distilled water instead of normal salt solution. The exposures were made at 37° C., while all others in this series were exposed at room temperature.

EXPERIMENT 6.

	Exposure	Death from Rabies		Exposure	Death from Rabies
Effect of 2 per cent phenol on virus suspension...........	1 day 2 days	Lived "	Effect of 0.85 per cent sodium chlorid on virus suspension..........	1 day 2 days 3 " 4 " 5 "	6th day 8th " 6th " 7th " Lived
Effect of 2 per cent sodium chlorid on virus suspension..	1 day 2 days	6th day Lived			
Effect of 0.4 per cent sodium chlorid and 0.4 per cent phenol on virus suspension..	1 day 2 days 3 " 4 "	6th day 7th " " " Lived			

It will be noted that a one per cent phenol does not destroy the virus during an exposure of six hours, while a two per cent solution kills it in less than 24 hours. A two per cent solution of sodium chlorid has a similar effect to that of the two per cent phenol, in that it destroys the virus in less than two days. In the higher percentages of sodium chlorid or phenol the effect on the virus is to be considered as due to the nonisotonic condition of the suspension rather than to a disinfectant action. When a one per cent suspension of fresh virus in a solution of sodium chlorid and phenol (0.4 per cent) is kept at 37° C. the virulence is not lost until the fourth day. This percentage of sodium chlorid and phenol will preserve the virulence of a five per cent fresh rabic brain, when kept in the cold room, for several weeks. On the other hand, most of the aldehyd compounds are extremely active in destroying the infectivity of fixed virus. A 0.5 per cent solution of acetaldehyd, benzaldehyd, formaldehyd or furfurol destroys the virus in less than three hours. A 0.5 per cent solution of salicylaldehyd, anisaldehyd or acetone has no effect up to an exposure of six hours. The specific disinfectant action of formaldehyd is shown by the fact that the virulence of the virus is lost when exposed for two hours to an 0.08 per cent solution. As a result of this work we advise, as a preliminary measure, the disinfection with formalin of all wounds made by animals suspected of being rabid.

The thermal death point of the virus is comparatively low; altho the results of heat tests vary somewhat with the same suspension under apparently the same conditions as to temperature and duration of exposure, yet these variations are not so great but that they fall within the range of experimental error. These experiments may be summed up as follows:

The virus is destroyed in 20 minutes at an exposure of 45° C.
" " " " " 15 " " " " 50° C.
" " " " " 5 " " " " 60° C.
" " " " " 2 " " " " 100° C.

Under certain conditions rabic virus readily passes through a tested Berkefeld filter, by a suction of 26 inches of mercury. This may be accomplished with great regularity if the rabic brain in suspension is not less than one per cent and is as nearly homogeneous as possible.

The accompanying table (p. 36) shows the results of two filtration experiments with the 1–25 suspension of rabic brain, one with the 1–70, and three with the 1–100 suspension.

It will be noted that the percentage of rabic brain in suspension influences the amount of virus in the filtrate. This is shown by determining the M.L.D. of the filtrate, and comparing this with the M.L.D. of the unfiltered suspension. For instance, the M.L.D.

of the unfiltered suspension is 0.5 c.c. of a 1–25,000 suspension of rabic brain, while the M.L.D. of the filtrate from the 1–25 suspension is represented by a dilution of approximately 1–1,000. This M.L.D. of the filtrate is one twenty-fifth that of the original suspension. Under similar conditions the M.L.D. of the filtrate from the 1–70 suspension is 0.5 c.c. of the 1–250 dilution. This dose represents one one-hundredth part of the virus in the unfiltered suspension. The undiluted filtrate from the 1–100 suspension was fatal in one out of three tests, and dilutions were not infectious.

Suspension of Rabic Brain	Filtrate Diluted to	Intracranial Injection of	Death from Rabies	Part of M.L.D.
1–25..............	1–800	0.5 c.c.	2 died	$\frac{1}{25}$
	1–1000	" "	1 " 1 lived	
	1–1200	" "	2 "	
1–70..............	1–200	" "	1 died	$\frac{1}{100}$
	1–250	" "	1 "	
	1–300	" "	1 lived	
1–100..............	1–100	" "	1 died 2 lived	$\frac{1}{250}$
	1–125	" "	3 "	
	1–150	" "	3 "	

When the undiluted filtrate is fatal the dose represents one two-hundred and fiftieth part of the virus in the unfiltered suspension. From these experiments it may be inferred: first, that rabic virus passes through a tested Berkefeld filter only under certain conditions as to homogeneity, dilution, and suction; second, that the amount of virus passing through the filter is inversely proportional to the dilution, other conditions being the same.

In considering the normal habitat of the virus, it is generally conceded that the mortality from inoculation depends upon the avenue of injection. For instance, in our experiments it was found that a single injection (1 c.c. of the standard suspension) into the lateral ear vein of a rabbit rarely infected. A series of these injections on three successive days usually terminated in death from rabies within four weeks. Intraperitoneal injections gave a mortality of about 15 per cent. The death rate among animals injected by the intramuscular or subcutaneous route was about 50 per cent. In contrast to the above extracranial injections those by the intracranial route resulted in a mortality of 100 per cent. By the extracranial methods it was noted that an injection with a needle of large caliber and roughened surface more often resulted fatally than an injection with a small smooth needle; also, serial injections almost invariably killed, whereas single injections usually gave a low mortality.

It is evident that infection depends upon the laceration and entrance of the virus into the nerve trunks. In the nervous system the virus has a suitable medium in which to reproduce itself. Here it is not exposed to the rapid interchange of the body fluids; consequently, when it gains access to the nerve trunks it readily extends its invasion. The antagonistic action of the blood and lymph contributes a favorable influence in reducing the mortality from hydrophobia. We can readily assume that the bite of a rabid animal results in the implantation of a variable quantity of the virus. The virus must come in direct contact with the broken nerve fibers in order to reproduce itself and to extend along the nerve trunks, to the spinal cord and to the brain. As long as it is limited to the peripheral nerves it cannot reproduce itself in sufficient quantities to cause symptoms of the disease. Upon its entrance, however, to the spinal cord and brain there is presented an extensive trunk system through which it readily spreads, and in which the virus reproduces itself in such quantities as to cause characteristic symptoms, and, finally, a fatal termination.

The foregoing statements must not be construed as meaning that, even after a lapse of three days, cauterization is valueless. The virus which is not in direct continuity with the broken nerve trunk will be destroyed by the blood and lymph; moreover, owing to the slow progress of the virus, a reopening and thorough scrubbing of the wound, followed by cauterization with formalin or with fuming nitric acid—even after a lapse of three days—will be of great value in reducing the quantity of infecting virus.

The action of the body fluids on rabic virus was tested as follows: collodion sacs containing the standard suspension were placed in the peritoneal cavity of either rabbits or guinea-pigs. While bacterial organisms are sacked for the purpose of increasing their virulence, our results with the rabic virus on the contrary, were the exact opposite. It was found that when the virus was exposed to the peritoneal fluid its virulence was lost in from three to six hours. The first sacking experiment consisted in introducing a sac into the peritoneal cavity of each of four rabbits. On each successive day following the operation one of the animals was anesthetized and the sac removed; 0.5 c.c. of its contents was injected intracranially into a guinea-pig. A portion of the original suspension used in this experiment was kept in the cold room, and injections from it served as control tests. On each successive day for four days a control animal was inoculated. All of the control animals died from rabies. On the other hand, none of those injected from the contents of the sacs succumbed to the disease. This experiment was verified by duplicate tests, and from these was proved the fact that the virus was destroyed in 24 hours. Moreover, subsequent experiments showed that the virus was no longer infectious when exposed to the body fluids for so short a time as four hours. In this set of experiments it was shown, by keeping the standard suspension at 37° C., that the body temperature alone does not destroy the infectivity of the virus for at least several days.

EXPERIMENT 1.

Exposure	Animal	Intracranial Inoculation	Death from Rabies
1 day	No. 1	0.5 c.c.	Lived
Control	" 2	" "	7th day
2 days	" 3	" "	Lived
Control	" 4	" "	6th day
3 days	" 5	" "	Lived
Control	" 6	" "	7th day
4 days	" 7	" "	Lived
Control	" 8	" "	8th day

This chart shows that exposure of the standard suspension to peritoneal fluid renders it non-virulent in one day. The control suspension was kept in the cold room.

EXPERIMENT 2.

Exposure	Animal	Intracranial Inoculation	Death from Rabies
2 hours	No. 1	0.5 c.c.	8th day
Control	" 2	" "	7th "
4 hours	" 3	" "	Lived
Control	" 4	" "	7th day
8 hours	" 5	" "	Lived
Control	" 6	" "	6th day
12 hours	" 7	" "	Lived
Control	" 8	" "	8th day

This chart shows that the virus, when exposed to the peritoneal fluid, is killed in at least four hours The control was kept at 37° C.

The following experiments show the effect of dialyzing a fresh standard suspension of rabic virus against running distilled water. These suspensions were dialyzed in collodion sacs prepared by the Novy method. In this series of experiments, as in all others, the only reliable method of showing the destruction of the virus is by the intracranial method of injection. Subcutaneous injection cannot be depended upon to show this destruction; as has already been pointed out, a virulent suspension is not always fatal when injected subcutaneously. At the termination of each series of exposure, an injection of the original untreated suspension served as a control.

EXPERIMENT 1.

Exposure	Guinea-Pig	Intracranial Injection	Death from Rabies
6 hours	No. 1	0.5 c.c.	6th day
12 "	" 2	" "	7th "
24 "	" 3	" "	6th "
Control	" 4	" "	" "

In this experiment a thick-walled collodion sac of 25 c.c. capacity was used.

EXPERIMENT 2.

Exposure	Guinea-Pig	Intracranial Injection	Death from Rabies
5 hours	No. 1	0.5 c.c.	7th day
10 "	" 2	" "	9th "
15 "	" 3	" "	7th "
24 "	" 4	" "	Lived
Control	" 5	" "	7th day

In Experiment 2 a sac of medium thickness with a capacity of 25 c.c. was employed.

EXPERIMENT 3.

Exposure	Guinea-Pig	Intracranial Injection	Death from Rabies
3 hours	No. 1	0.5 c.c.	8th day
6 "	" 2	" "	7th "
9 "	" 3	" "	8th "
12 "	" 4	" "	Lived
Control	" 5	" "	7th day

In Experiment 3 an extremely thin sac of 40c.c. capacity was used.

EXPERIMENT 4.

Exposure	Guinea-Pig	Intracranial Injection	Death from Rabies
1 day	No. 1	0.5 c.c.	6th day
2 days	" 2	" "	7th "
3 "	" 3	" "	6th "
4 "	" 4	" "	8th "
Control	" 5	" "	" "

In Experiment 4 a three per cent suspension was dialyzed in a medium thick sac of 40 c.c. capacity.

EXPERIMENT 5.

Exposure	Guinea-Pig	Intracranial Injection	Death from Rabies
5 hours	No. 1	0.5 c.c.	7th day
10 "	" 2	" "	" "
15 "	" 3	" "	8th "
24 "	" 4	" "	7th "
Control	" 5	" "	" "

In Experiment 5 a very thin walled sac containing 40 c.c. of suspension was suspended in a cylinder containing an equal volume of distilled water.

EXPERIMENT 6.

Exposure	Guinea-Pig	Intracranial Injection	Death from Rabies
5 hours	No. 1	0.5 c.c.	8th day
10 "	" 2	" "	7th "
15 "	" 3	" "	8th "
24 "	" 4	" "	Lived
Control	" 5	" "	7th day

In Experiment 6 the same sac employed in Experiment 5 containing 40 c.c. of suspension was dialyzed against running distilled water.

It will be noted from these experiments that the thickness of the sac, the percentage of rabic brain in suspension, and the rate of flow of distilled water are important factors in effecting a destruction of the virus.

Our investigation of the protective value of the different methods of antirabic treatment consisted, first, in determining the M.L.D. of the fixed virus; second, in treating numerous sets of animals (rabbits and guinea-pigs) by the various methods, which are outlined in detail below; third, in subjecting these sets, after an interval of from two weeks to six months, to immunity tests.

In determining the M.L.D. of the fixed virus the following procedure of preparation is vital, inasmuch as it is essential to use from day to day fresh suspensions of uniform homogeneity. These suspensions were made from rabic brain obtained from stock rabbits immediately after death. The brain tissue was brought into suspension in the following manner: one gram of tissue and an equal volume of fine sharp sand were thoroughly crushed for five minutes with a heavy glass rod in a 150 c.c. test-glass. Then a few drops of physiological salt solution were added, and the mixture stirred for two minutes so as to bring the tissue into a thick suspension. After this two minutes of continual stirring, small quantities of salt solution were added, these additions alternating with mixing. Finally, larger quantities were added and stirred until there was a total of 100 c.c. The suspension, thus prepared, was kept in the cold room for 24 hours in order to free the supernatant liquid from lumps. About

75 c.c. of the supernatant suspension were then drawn off with a bulb pipette and filtered through a No. 595 Schleicher and Schüll filter paper. From this standard homogeneous filtrate (presumably 1–100) higher dilutions were made and injected. The procedure of preparing this standard suspension must be unvarying from day to day, so that a fixed quantity of a certain dilution of the suspension will represent the M.L.D.; should this dose vary from time to time no reliance can be placed on immunity tests, and such variations would indicate inaccuracy in preparing the basic standard 1–100 suspension.

Attempts were first made to determine the M.L.D. by the subcutaneous method of injection. From the standard 1-100 suspension, dilutions up to 1–500 of rabic brain were made which represented 1–200, etc. Of these suspensions one cubic centimeter was injected subcutaneously. Six sets of five guinea-pigs each were injected respectively with the standard suspension and its dilutions. In no two of these sets was the M.L.D. represented by the same dilution. In fact the 1–100 suspension was not always fatal; yet in the same set a higher dilution did result in death. Suspensions of 1–10, 1–20, etc., up to 1-100 also gave irregular results. It was, indeed, found that the 1–10, altho not always fatal itself, would, when diluted, produce death. A series of daily injections of dilutions up to and including 1–100 for three days invariably terminated in death. By this avenue of injection the incubation period was from 18 to 72 days. Intramuscular injections gave results very similar to the subcutaneous ones. By both of these methods of injections the M.L.D. could not be accurately determined; these, therefore, were not adopted in making immunity tests.

Realizing that the nerve tissue is the normal habitat of the virus, an attempt was now made to ascertain the M.L.D. by the intracranial method of injection. From a freshly prepared standard suspension, gradually decreasing doses were made by dilution; 0.5 c.c. of each dilution was injected intracranially. It was found that all injections of dilutions up to 1–20,000 were fatal; at times as high a dilution as 1–25,000 would kill. For the purpose of making immunity tests it was desirable to fix this M.L.D. within more narrow limits. At this point in the work it was found that the virus obtained from a rabbit which had died after several days of complete paralysis was not as virulent as that taken from one at the beginning of this stage. The rabic brain tissue at the beginning of the complete paralytic stage is not only more virulent, but its virulence is more uniform than are specimens taken after death. The animal may be killed at the beginning of this stage with ether, or by anesthesia and bleeding. The brain is then immediately removed and a standard 1–100 suspension is made as directed above. Suspensions of rabic brain made from rabbits killed in this stage were found—as the method of preparation was perfected—to be fatal in dilutions up to and almost invariably including 1–25,000 when injected intracranially in doses of 0.5 c.c. into rabbits and guinea-pigs. Numerous attempts to infect with a dilution of 1–26,000 failed; on the other hand an injection of 1–23,000 was occasionally the highest fatal dilution.

Having determined the M.L.D. of the fixed virus by the intracranial method of injection, the next step in demonstrating the protection afforded by the various methods of antirabic treatment was to immunize numerous sets of animals by these methods.

In making the immunity tests several sets of animals each of which had been immunized by one or another of the three methods were in some instances tested at the same time; the same dilutions, or subdilutions from the original 1–100 suspension were used in all sets tested. The purpose of such a procedure was to have the test conditions the same for the different sets. According to this method Tests 1 and 4 of the Pasteurian series were carried out at the same time as Tests 1, 3, and 4 of the Dialyzed series; Test 3 of the Pasteur and Test 4 of the Högyes; Test 3 of the Högyes and Test 21 of the Dialyzed; Test 1 of the Högyes and Tests 4 and 7 of the Dialyzed; Tests 2, 5, and 6 of the Pasteur and Tests 6, 12, 13, 15, and 19 of the Dialyzed series.

In the Högyes or the dilution method of treatment there were first prepared suspensions of 1–100 of fresh rabic brain in normal salt solution. In making these suspensions and their dilutions the procedure was that followed in working out the M.L.D.; there was, however, one point of difference: the animals were permitted to die. The dilutions and dosage on successive days are indicated as follows:

Day	Dilution	Dose c.c.	Day	Dilution	Dose c.c.
1st – 3d	1–10,000	4	18th	1–3,000	2
4th– 6th	1– 8,000	4	19th	1–2,000	2
7th– 9th	1– 6,000	4	20th	1–1,000	2
10th–13th	1– 5,000	2	21st	1– 500	2
14th–17th	1– 4,000	2			

The following tabulations show the immunity tests on four sets of animals treated with the above dilutions.

IMMUNITY TEST 1.

Animal	Dose	Death from Rabies
No. 1	1.65[*] M.L.D.	7th day
" 2	1.35 "	Lived
" 3	1.1 "	"
Control	1.0 "	9th day

Interval between treatment and test, 45 days.

IMMUNITY TEST 2.

Animal	Dose	Death from Rabies
No. 1	2.5 M.L.D	7th day
" 2	1.75 "	Lived
" 3	1.65 "	"
Control	1.0 "	6th day

Interval between treatment and test, 78 days.

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[*] In all tabulations 1 M.L.D. represents 0.5 c.c. of a 1–25,000 dilution of rabic brain in physiological salt solution. 1 M.L.D. is equal to 0.02 milligram of brain tissue. 1.65 M.L.D. equals 0.5 c.c. of 1–15,000 dilution of brain tissue. The 0.5 c.c. of 1–15,000 dilution is equal to 0.033 milligram or 1.65 M.L.D.

IMMUNITY TEST 3.

Animal	Dose	Death from Rabies
No. 1	2.5 M.L.D.	9th day
" 2	1.85 "	Lived
" 3	1.65 "	"
Control	1.05 "	7th day

Interval between treatment and test, 93 days.

IMMUNITY TEST 4.

Rabbit	Dose	Death from Rabies
No. 1	3.0 M.L.D.	10th day
" 2	2.2 "	" "
" 3	1.75 "	" "
" 4	1.4 "	Lived
" 5	1.25 "	"
Control	1.05 "	6th day
"	1.2 "	8th day

Interval between treatment and test, 45 days.

SUMMARY OF ABOVE TABULATIONS.

Dose	Animals Tested	Death from Rabies	Protected
3.0 M.L.D	1	1	
2.5 "	2	2	
2.2 "	1	1	
1.85 "	1		1
1.75 "	2	1	1
1.65 "	3	1	2
1.4 "	1		1
1.35 "	1		1
1.25 "	1		1
1.1 "	1		1

It will be noted in the summary of the above tabulations that the immunity conferred by this method of treatment will protect against about 1.5 M.L.D., and as will be shown, it is less than by the original method of Pasteur. Furthermore, an intracranial injection of any one of the dilutions used in the Högyes treatment will produce the disease in rabbits or guinea-pigs.

A modification of the above method, as used by some workers, consists in the use of freshly prepared virus—presumably 1–100—a method which is positively dangerous. The experience of thus inoculating the patient is demonstrated by Breggi. Attempts to produce immunity in rabbits and guinea-pigs in this laboratory with the 1–100 suspension resulted in infection from the treatment itself. In numerous sets of five animals so treated, infection resulted in two or more animals; at times, even all the animals in a given set died.

As carried out in this laboratory the Pasteur treatment consisted of injections of suspensions made from 14-day to 4-day desiccated rabic spinal cords. These suspensions were made in the following manner: From three-eighths to one-half of an inch of the required cord was transferred to a test-glass containing sharp sand, and thoroughly crushed with a glass rod. A few drops of normal salt solution were then added, and a thick suspension was made by continuing the crushing process. At intervals more salt solution was added, the additions alternating with mixing until a total of 30 c.c. was added. By following this procedure a thoroughly homogeneous suspension is obtained. The spinal cord suspensions and the dosage were as follows:

Day	Spinal Cord	Dose c.c.	Day	Spinal Cord	Dose c.c.
1st	14–13	6	12th	6	2
2d	12–11	6	13th	5	2
3d	10–9	6	14th	4	2
4th	8	2	15th	6	2
5th	7	2	16th	5	2
6th	6	2	17th	4	2
7th	5	2	18th	6	2
8th	4	2	19th	5	2
9th	6	2	20th	4	2
10th	5	2	21st	4	2
11th	4	2	....	....	....

The following tabulations show the immunity tests on six sets of animals treated by the Pasteur method:

TEST 1.

Interval: 43 Days.

Dose	Guinea-Pig	Death from Rabies
3.0 M.L.D	No. 1	7th day
2.5 "	" 2	Lived
2.05 "	" 3	7th day
1.65 "	" 4	Lived
1.5 "	" 5	"
1.05 "	Control	7th day

TEST 2.

Interval: 51 Days.

Rabbit	Death from Rabies
....	....
....	....
No. 1	8th day
" 2	52d "
....	....
Control	6th "

TEST 3.

Interval: 60 Days.

Dose	Rabbit	Death from Rabies
3.0 M.L.D	....	....
2.5 "	No. 1	8th day
2.05 "	" 2	" "
1.75 "	" 3	Lived
1.4 "	" 4	"
1.0 "	Control	7th day

TEST 4.

Interval: 68 Days.

Guinea-Pig	Death from Rabies
....	....
No. 1	8th day
" 2	" "
" 3	Lived
" 4	"
Control	8th day

TEST 5.

Interval: 101 Days.

Dose	Guinea-Pig	Death from Rabies
6.0 M.L.D	....	....
3.0 "	No. 1	11th day
2.5 "	" 2	10th "
1.5 "	" 3	Lived
1.25 "	Control	9th day
1.05 "	"	" "

TEST 6.

Interval: 180 Days.

Guinea-Pig	Death from Rabies
No. 1	8th day
" 2	Lived
" 3	9th day
" 4	Lived
....	....
Control	8th day

SUMMARY OF ABOVE TABULATIONS.

Dose	Animals Tested	Death from Rabies	Protected
6.0 M.L.D	1	1	......
3.0 “	4	4	......
2.5 “	3	2	1
2.05 “	6	5	1
1.75 “	2	......	2
1.65 “	2	1	1
1.5 “	3	......	3
1.4 “	1	......	1

These tabulated experiments are the last 6 of a series of 17 which were carried out in our investigation of the protection afforded by the Pasteur treatment. The last 6 are presented owing to the fact that in these the results are more reliable than in the earlier numbers, inasmuch as the technic of testing was perfected as the series progressed.

In the summary it will be observed that the Pasteur treatment protects against about 2 M.L.D. of the fixed virus injected by the intracranial method. The immunity produced by this method of treatment may be expected to protect against an amount of street virus subcutaneously introduced equivalent to 2 M.L.D. of fixed virus injected intracranially, or a smaller amount which, during an interval of exposure, has increased to an equivalent of 2 M.L.D. We may then say that a case is hopeless if the street virus introduced into the wound is equal to 3 M.L.D., or if a small dose of the original virus multiplies to 3 M.L.D. during an interval of exposure.

The vital preliminary to the vaccine treatment is the proper cauterization of the wound; a second, and equally important step, is immediate antirabic treatment. If in a severe lacerating bite an equivalent of 3 M.L.D. of the fixed virus, injected intracranially, is introduced into the wound, one-half or even more of the infecting virus may be destroyed by proper cauterization, and the subsequent preventive injections will protect against that remaining. The value of cauterization with formalin, especially in face bites and lacerating ones on other parts of the body, cannot be overestimated. This, followed by the vaccine treatment, should reduce the mortality from hydrophobia to zero.

In addition to the work on the Högyes and the Pasteur methods of antirabic treatment we present the experimental and practical results of a method which developed from our original research with rabic virus in this laboratory. For the purpose of designation this will be termed the method of dialysis. The destructive action effected by dialyzing the virus against running distilled water has already been explained. The preparation of the vaccine by this method is based on the use of a thoroughly homogeneous suspension of rabic brain and the employment of standardized collodion sacs. The technic of preparing the homogeneous suspension is the same as that given in detail under the discussion of the M.L.D.; there are, however, two points of exception: (1) in place of physiological salt solution, distilled water is used; (2) the animal is not killed at the beginning of the complete paralytic stage, but is permitted to die from the disease.

In the standardization of collodion sacs the procedure is as follows: Thin sacs are prepared by the Novy method. They are sterilized in the autoclav at 105° C. for 20 minutes. These are then filled to the glass line with the standard suspension which is dialyzed against running distilled water. At intervals of 8, 16, and 24 hours a few cubic centimeters are withdrawn from each sac, and 0.5 c.c. of each specimen is injected intracranially into a guinea-pig. After the injection from the 24-hour exposure has been made the contents of the sacs are withdrawn. They are then filled with 0.4 per cent formaldehyd solution, and set aside for future use awaiting the results of the animal inoculations. At the termination of 10 days the results of these inoculations indicate the rate of dialysis in the various sacs. If in any case the injection from the 24-hour exposure caused death from rabies in the test animal, the sac in which this suspension had been dialyzed was discarded. On the other hand, those sacs in which the virus was destroyed within 24 hours were retained and made use of in preparing further quantities of vaccine.

A similar method which does not require such a prolonged dialysis is that of first destroying the active virulent virus by the use of any of the specific disinfectants. For this purpose the standard homogeneous suspension in distilled water is treated with 0.1 per cent formaldehyd. After sedimentation for three hours the supernatant liquid is drawn off and dialyzed until the test for formaldehyd becomes negative.

The vaccine obtained by either of these methods of dialysis is tested as follows: first, by intracranial injections into guinea-pigs, for both infectivity of the virus and bacterial contamination; second, on artificial media for bacterial contamination. As a preservative 0.1 per cent tricresol is added.

Of the following 25 immunity tests those animals in Tests 1, 2, 11, 17, 18, and 25 were immunized with vaccine which was first treated with formaldehyd and then dialyzed, whereas those in the remaining Tests (3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 19, 20, 21, 22, 23, and 24) were immunized with vaccine prepared by dialysis alone.

The accompanying tabulations are arranged according to the immunizing dose; the number of days treated, and the interval elapsing between the treatment and the immunity tests. The sets of animals receiving small doses for a brief time and having a short interval appear first in the series.

TEST 1.

Dose	Guinea-Pig*	Death from Rabies
3.0 M.L.D.	No. 1	Lived
2.5 "	" 2	"
2.05 "	" 3	"
1.75 "		
1.60 "	" 4	"
1.30 "	" 5	"
1.20 "		
1.05 "	Control	7th day
1.0 "		

TEST 2.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
" 4	"
" 5	"
Control	8th day
"	7th "
"	10th "

* Protective treatment: 2 c.c. dialyzed vaccine on 11 successive days. Interval: 43 days.

† Protective treatment: 2 c.c. dialyzed vaccine on 11 successive days. Interval: 105 days.

TEST 3.

Dose	Guinea-Pig*	Death from Rabies
4.0 M.L.D.	No. 1	Lived
3.0 "	" 2	8th day
2.5 "	" 3	Lived
2.05 "	" 4	"
1.75 "	" 5	"
1.65 "		
1.4 "	" 6	"
1.0 "	Control	7th day

TEST 4.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
" 4	"
Control	7th day

* Protective treatment: 2 c.c. of dialyzed vaccine (which had been kept in the cold room for five months) on 12 successive days. Interval: 57 days.

† Protective treatment: 2 c.c. of dialyzed vaccine (which had been kept in the cold room for three months) on 14 successive days. Interval: 48 days.

TEST 5.

Dose	Guinea-Pig*	Death from Rabies
6.0 M.L.D.		
4.0 "		
3.0 "		
2.5 "	No. 1	Lived
1.65 "	" 2	"
1.35 "	" 3	"
1.05 "		
1.0 "	Control	8th day

TEST 6.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
Control	9th day

* Protective treatment: 3 c.c. dialyzed vaccine (1 part rabic brain to 25 parts water) on 9 successive days. Interval: 37 days.

† Same animals as in Test 5, tested again after an interval of 120 days.

TEST 7.

Dose	Guinea-Pig*	Death from Rabies
4.0 M.L.D		
3.0 "		
2.5 "	No. 1	Lived
1.6 "	" 2	"
1.05 "		
1.0 "	Control	8th day

TEST 8.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
Control	9th day

* Protective treatment: 3 c.c. dialyzed vaccine on 10 successive days. Interval: 37 days.

† Test 7 animals tested again after an interval of 120 days.

TEST 9.

Dose	Guinea-Pig*	Death from Rabies
3.0 M.L.D	No. 1	Lived
2.5 "	" 2	2d day‡
2.05 "		
1.65 "	" 3	Lived
1.35 "	" 4	"
1.0 "	Control	7th day

TEST 10.

Guinea-Pig†	Death from Rabies
No. 1	10th day
" 2	Lived
" 3	"
Control	7th day

* Protective treatment: 3 c.c. dialyzed vaccine (which had been kept in the cold room for 3 months) on 14 successive days. Interval: 58 days.

† These are the remaining 3 animals from Test 9 tested again 25 days later.

‡ Cultures from heart's blood showed a bacillus.

TEST 11.

Dose	Guinea-Pig*	Death from Rabies
6.0 M.L.D		
4.0 "	No. 1	Lived
3.0 "	" 2	9th day
2.5 "	" 3	Lived
2.0 "	" 4	"
1.75 "	" 5	"
1.5 "	" 6	"
1.0 "	Control	8th day

TEST 12.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
Control	8th day

* Protective treatment: 3 c.c. dialyzed vaccine (which had been kept in the cold room for 3 months), on 14 successive days. Interval: 72 days.

† Protective treatment: 4 c.c. dialyzed vaccine, intraperitoneally, on 16 successive days. Interval: 120 days.

TEST 13.

Dose	Guinea-Pig*	Death from Rabies
3.5 M.L.D		
3.0 "	No. 1	Lived
2.5 "		
2.05 "	" 2	"
1.5 "	" 3	"
1.02 "	Control	7th day

TEST 14.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
Control	7th day

* Protective treatment: 5 c.c. dialyzed vaccine, injected intraperitoneally, on 7 successive days. Interval: 60 days.

† Animals in Test 13 re-tested 40 days later.

TEST 15.

Dose	Rabbit*	Death from Rabies
5.0 M.L.D.		
3.5 "		
3.0 "	No. 1	Lived
2.8 "		
2.05 "	" 2	"
1.5 "	" 3	"
1.05 "	Control	7th day

TEST 16.

Rabbit†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
Control	7th day

* Protective treatment: 5 c.c. dialyzed vaccine on 7 successive days. Interval: 20 days.

† Animals in Test 15 re-tested 40 days later.

TEST 17.

Dose	Guinea-Pig*	Death from Rabies
3.0 M.L.D.	No. 1	62nd day
2.5 "	" 2	Lived
1.95 "	" 3	"
1.65 "	" 4	"
1.35 "	" 5	"
1.0 "	Control	7th day

TEST 18.

Guinea-Pig†	Death from Rabies
No. 1	7th day
" 2	Lived
" 3	"
" 4	"
" 5	8th day
Control	7th "

* Protective treatment: 5 c.c. dialyzed vaccine subcutaneously on 8 successive days. Interval: 20 days.

† Protective treatment: same as Test 17 except that the injections were made intraperitoneally. Interval: 20 days.

TEST 19.

Dose	Guinea-Pig*	Death from Rabies
8.0 M.L.D.		
6.0 "	No. 1	Lived
3.5 "	" 2	"
2.05 "	" 3	"
1.0 "	Control	8th day

TEST 20.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
Control	7th day

* Protective treatment: 5 c.c. dialyzed vaccine intraperitoneally on 8 successive days. Interval: 90 days.

† Animals in Test 19 re-tested 40 days later.

TEST 21.

Dose	Guinea-Pig*	Death from Rabies
3.0 M.L.D.		
2.5 "	No. 1	Lived
2.05 "	" 2	"
1.75 "	" 3	"
1.05 "	Control	8th day
1.0 "		

TEST 22.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
Control	8th day

* Protective treatment: 5 c.c. dialyzed vaccine (which had been kept in the cold room for 10 weeks) intraperitoneally on 9 successive days. Interval: 70 days.

† Animals in Test 21 re-tested 150 days later.

TEST 23.

Dose	Guinea-Pig*	Death from Rabies
3.0 M.L.D	No. 1	Lived
2.0 "	" 2	"
1.35 "	" 3	"
1.0 "	Control	7th day

TEST 24.

Guinea-Pig†	Death from Rabies
No. 1	Lived
" 2	"
" 3	"
Control	7th day

* Protective treatment: 5 c.c. dialyzed vaccine intraperitoneally on 15 successive days. Interval: 44 days.

† Protective treatment: same as Test 23 except that the injections were made subcutaneously. Interval: 44 days.

TEST 25.

Dose	Guinea-Pig*	Death from Rabies
6.0 M.L.D	No. 1	Lived
3.0 "	" 2	"
2.05 "	" 3	"
1.05 "	Control	8th day

* Protective treatment: 5 c.c. dialyzed vaccine intraperitoneally on 16 successive days. Interval 120 days.

SUMMARY OF THE ABOVE TABULATIONS.

Dose	Animals Tested	Death from Rabies	Protected
8 M.L.D	1		1
6 "	5		5
5 "	1		1
4 "	8		8
3.5 "	1		1
3 "	19	5*	14
2.8 "	1		1
2.5 "	15		15
2.05 "	9		9
2 "	3		3
1.95 "	2		2
1.75 "	4		4
1.65 "	4		4
1.60 "	4		4
1.5 "	3		3
1.4 "	2		2
1.35 "	6	1†	5
1.3 "	2		2

* These deaths may be partially explained by reason of the fact that two of the series were treated with old vaccine (2 and 5 months old), and in two others the interval between treatment and immunity tests was comparatively short.

† Death of this animal is apparently an example of no response to treatment.

It will be noted in the summary of the above tests that this method of treatment will protect against as much as 8 M.L.D. Furthermore, the vaccine so prepared, when injected intracranially, will not produce the disease; therefore, subcutaneous injections of the dialyzed vaccine are not only perfectly safe as a prophylactic measure, but, as has been shown, they confer the highest degree of immunity. It is possible to reproduce rabies in animals by intracranial injection of a suspension made from a six-day desiccated cord, and an intracranial injection of any one of the dilutions used in the Högyes treatment will result in death from rabies. By this new method, however, of preparing the vaccine, it is impossible to produce the disease in animals even by intracranial injections, thus absolutely eliminating all danger of infection by subcutaneous injections, while at the same time, this method confers the highest degree of immunity that can be obtained with safety. A series of 12 successive daily injections of the standard dialyzed vaccine gives the same degree of immunity as is obtained by the intensive form of the Pasteur treatment, which consists of 21 daily injections. The vaccine prepared by the method of dialysis is of especial value, not only in the case of severe bites, particularly those on the face, but also in those cases which do not report for treatment earlier than two weeks after having been bitten.

In conclusion it may be stated that, in this Institute and elsewhere in the United States, over 800 persons bitten by animals suspected of having rabies were treated by this new method. Of this number 62 per cent were bitten by animals proved by laboratory examination to have been rabid. In this series of patients the treatment has been efficient in protecting against hydrophobia in every case. The treatment consisted of two cubic centimeters daily for from 15 to 25 days. A local anaphylactic reaction at the point of injection is not uncommon among patients treated by the dialyzed vaccine. It manifests itself on the seventh or eighth day by redness, slight swelling, and itching. In no case is the reaction marked, and its duration is about 24 hours. This phenomenon is not due to rabic virus, or its products of cleavage, but is common to a series of injections of any foreign proteins—in this case brain cell protein. In cases of trivial scratches and those in which there was a possibility of the saliva having entered an open wound a 15-day course of treatment was considered sufficient. In the average case from 18 to 21 daily treatments were prescribed; whereas in cases of severe uncauterized bites on uncovered surfaces of the body, especially on the face, and in which there had been a delay of two weeks or more in applying for treatment it was considered desirable to give two treatments daily for the first four days, and these were followed by single daily injections through the twenty-fifth day.

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